

In one embodiment, this invention provides a nucleic acid encoding the carboxy-terminal portion of the heavy chain (HC) of botulinum neurotoxin (BoNT), the BoNT being selected from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype C1, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G, wherein said nucleic acid is expressable in a recombinant organism selected from *Escherichia coli* and *Pichia pastoris*. Preferably, the nucleic acid comprises a nucleic acid sequence selected from SEQ ID No:1 (serotype A), SEQ ID No:7 (serotype B), SEQ ID No:9 (serotype C1), SEQ ID No:11 (serotype D), SEQ ID No:13 (serotype E), SEQ ID No:15 (serotype F), and SEQ ID No:17 (serotype G). In an alternative preferred embodiment, the nucleic acid encodes an HC amino acid sequence of BoNT selected from SEQ ID No:2 (serotype A), SEQ ID No:8 (serotype B), SEQ ID No:10 (serotype C1), SEQ ID No:12 (serotype D), SEQ ID No:14 (serotype E), SEQ ID No:16 (serotype F), and SEQ ID No:18 (serotype G).

In another embodiment, this invention provides a nucleic acid encoding the amino-terminal portion of the heavy chain (HN) of botulinum neurotoxin (BoNT), the BoNT being selected from the group consisting of BoNT serotype B, BoNT serotype C1, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G, wherein said nucleic acid is expressable in a recombinant organism selected from *Escherichia coli* and *Pichia pastoris*. In a preferred embodiment, the nucleic acid comprises a nucleic acid sequence selected from SEQ ID No:21 (serotype B), SEQ ID No:23 (serotype C1), SEQ ID No:25 (serotype D), SEQ ID No:27 (serotype E), SEQ ID No:29 (serotype F), and SEQ ID No:31 (serotype G). Alternatively, the nucleic acid encodes an HN amino acid sequence of BoNT selected from SEQ ID No:22 (serotype B), SEQ ID No:24 (serotype C1), SEQ ID No:26 (serotype D), SEQ ID No:28 (serotype E), SEQ ID No:30 (serotype F), and SEQ ID No:32 (serotype G).

Preferably, the nucleic acid of this invention is a synthetic nucleic acid. In a preferred embodiment, the sequence of the nucleic acid is designed by selecting at least a portion of the codons encoding HC from codons preferred for expression in a host organism, which may be selected from gram negative bacteria, yeast, and mammalian cell lines; preferably, the host organism is *Escherichia coli* or *Pichia*

weight markers. Lane 2 is the cell lysate, lane 3 is the cell extract, lane 4 is the cell extract after dialysis, lane 5 is pool of rBoNTF(Hc) positive fractions after Mono S column chromatography, and lane 6 is pool of rBoNTF(Hc) positive fractions after hydrophobic interaction chromatography.

Figure 21 shows purification of rBoNTF(Hc) by sequential chromatography. (A) Mono S cation exchange chromatography of extract from *P. pastoris*. Proteins were eluted with increasing NaCl gradient. Fractions positive for rBoNTF(Hc) by Western analysis were pooled individually and subjected to hydrophobic interaction chromatography (B) and proteins were eluted with a decreasing ammonium sulfate gradient. In both panels, protein monitored by A280nm is recorded on the left axis and elution conditions are recorded on the right axis, with the gradient trace laid over the chromatogram.

Figure 22 shows CD spectra of purified soluble (—) and resolubilized (--) rBoNTF(Hc) at 30 µg/ml (0.62 µM) in 10 mM sodium phosphate, pH 7.0 in a 1-cm path length cell. Spectra were the average of four accumulations, scanned from 260 to 200 nm at a scan rate of 10 nm/min with a 2-s response and a 1-nm bandwidth. The temperature was maintained at 20°C using a Peltier thermocontrol device.

DETAILED DESCRIPTION OF THE EMBODIMENTS OF THIS INVENTION

The present inventors have determined that animals, including primates, may be protected from the effects of botulinum neurotoxin (BoNT) by immunization with fragments of the botulinum neurotoxin protein expressed by recombinant organisms. Specifically, peptides comprising protective epitopes from the receptor binding domain and/or the translocation domain, found in the carboxy terminal and the amino terminal portions of the heavy chain of the BoNT protein, respectively, are expressed by recombinant organisms transfected with expression vectors encoding the peptides for each serotype of BoNT. Immunization with these recombinantly produced peptides will elicit antibodies capable of protecting animals against intoxication with the BoNT of the respective serotype.

This invention provides a genetically engineered vaccine for protection against botulism. The vaccine comprises fragments of the A and B toxins known as

Synthetic gene construction is a technique used to optimize for expression in heterologous host systems. The base composition (i.e., percent A+T or percent G+C) as well as the specific codons in a gene sequence play a role in determining whether a gene from one organism will be optimally expressed in a different organism. There is a reason why certain codons are used and why some are not. Organisms will use the codons in which corresponding tRNAs are present. If the organisms do not use certain codons, they most likely lack those specific tRNAs. As it turns out, codons found in clostridial DNA (i.e., genes found in the genus of bacterial called *Clostridium*) are very unique both in terms of base composition (i.e., very high A+T base composition) and in the use of codons not normally found in *E. coli* or yeast.

Table 1 is a chart depicting codon usage in *Pichia pastoris*. This table was generated by listing the codons found in a number of highly expressed genes in *P. pastoris*. The codon data was obtained by sequencing the genes and then listing which codons were found in the genes.

From Table 1, it is clear that the amino acid residues can be encoded for by multiple codons. When constructing synthetic genes using *P. pastoris* codon usage, it is preferred to use only those codons that are found in the naturally occurring genes in *P. pastoris*, and it should be attempted to keep them in the same ratio found in the genes of the natural organism. When the clostridial gene has an overall A+T richness of greater than 70% and A+T regions that have spikes of A+T of 95% or higher, they have to be lowered for expression in expression systems like yeast. (Preferably, the overall A+T richness is lowered below 60% and A+T in spikes is also lowered to 60% or below). It is of course necessary to balance keeping the same codon ratio (e.g., for glycine GGG was not found, GGA was found 22% of the time, GGT was found 74% of the time, GGC was found 3% of the time) with reducing the high A+T content. In the construction of the genes, it is preferred to keep the A+T spikes about 55%.

Considering codon usage for a number of organisms including *E. coli*, it turns out that a synthetic gene using *E. coli* codon usage also expresses fairly well in *P. pastoris*. Similarly, a synthetic gene using *P. pastoris* codon usage also appears to express very well in *E. coli*.